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Invited Mini Review

Water Transport across the Peritoneal Membrane

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ABSTRACT
Peritoneal dialysis involves diffusive and convective transports and osmosis through the highly vascularized peritoneal membrane. The capillary endothelium offers the rate-limiting hindrance for solute and water transport. It can be functionally described in terms of a threepore model including transcellular, ultrasmall pores responsible for free-water transport during crystalloid osmosis. Several lines of evidence have demonstrated that the water channel aquaporin-1 (AQP1) corresponds to the ultrasmall pore located in endothelial cells. Studies in *Aqp1* mice have shown that deletion of AQP1 is reflected by a 50% decrease in ultrafiltration and a disappearance of the sodium sieving. Haplo-insufficiency in AQP1 is also reflected by a significant attenuation of water transport. Conversely, studies in a rat model and in PD patients have shown that the induction of AQP1 in peritoneal capillaries by corticosteroids is reflected by increased water transport and ultrafiltration, without affecting the osmotic gradient and small solute transport. Recent data have demonstrated that a novel agonist of AQP1, predicted to stabilize the open state conformation of the channel, modulates water transport and improves ultrafiltration. Whether increasing the expression of AQP1 or gating the already existing channels would be clinically useful in PD patients remains to be investigated.

The development of peritoneal dialysis (PD) as a successful therapy for patients with end stage renal disease has been paralleled by the need to understand the transport mechanisms operating in the peritoneal membrane. Functional alterations in the dialysis capacity of the membrane, leading to ultrafiltration failure, are associated with increased morbidity and mortality and represent a major obstacle to successful long-term PD therapy (1). Over the past three decades, investigations based on human and animal models provided significant insights into the physiology of transport pathways for water and solutes across the peritoneal membrane. Subsequent development of genetically modified mouse models yielded further insights and, in particular, molecular counterparts involved in peritoneal water transport (2). The purpose of this brief review is to discuss the current understanding of water transport mechanisms operating in the peritoneal membrane, and to delineate potential strategies to improve water transport and ultrafiltration in patients on PD.

1. Structure of the peritoneal membrane

The peritoneal cavity is lined by a thin, translucent membrane covering the inner surface of the abdominal wall and the majority of visceral organs, and also forming omenta. The peritoneal membrane (Fig. 1A) has a relatively large surface area (~1 m^2) (3), a high degree of capillarization, and a relatively high blood flow (100-150 mL/min) in adults (4-6). The compact zone of the visceral peritoneum (that forms most of the peritoneal surface area) is about 20 ⌈m-thick in PD patients, whereas the parietal peritoneum can be thickened up to 500 ⌈m in long-term PD patients, compared to 50 ⌈m in controls (7).
The blood capillaries are distributed in a thin interstitium where their density is relatively high: for 1 m^2 of peritoneal surface area and 200 ⌈m tissue depth, the total available capillary surface area is <2 m^2 (8). The surface of the peritoneal membrane is lined by a

continuous layer of flattened epithelial cells, the mesothelium (0.5-2 ⌈m thickness), covered with numerous microvilli. The mesothelium reduces the friction between visceral organs through the continuous production of lubricants, anticoagulants and surface tension lowering substances (8).

The pathways available for solute and water exchange between the plasma in the peritoneal capillaries and the fluid in the peritoneal cavity include i) the continuous capillary endothelium; ii) the peritoneal interstitial space; and iii) the mesothelium. Of these potential barriers, it is the capillary endothelium that seems to offer the rate-limiting hindrance, restricting the solute exchange to less than 0.1% of its total surface area (4,8-10). Solutes larger than glucose are hindered in their transport across the permeable pathways ("pores") of the capillary wall. The interstitium may also modify the transport of solutes across the peritoneal barrier, accounting for approximately 20-30% of the total diffusion resistance for a small solute (11).

In the capillary wall (Fig. 1A, inset), the major route for small solute and fluid exchange is represented by the spaces in-between individual endothelial cells, the so-called interendothelial clefts (10). The functional radius of these clefts, denoted "small pores", is ~40-50 Å, i.e. slightly larger than the radius of albumin (36 Å). The small pores markedly impede the transit of albumin (by at least three orders of magnitude) and completely prevent the passage of larger molecules, such as immunoglobulins and ⟨2-macroglobulin, across the capillary endothelium. However, large proteins can permeate to the interstitium (and the peritoneal cavity) via very rare transendothelial "large pore" pathways (radius ~250 Å) (12). These large pores account for only 0.01% of the total population of capillary pores and transport across them occurs by hydrostatic pressure-driven unidirectional filtration from the plasma to the peritoneal cavity. In addition, the capillary wall has a high permeability to osmotic water transport through ultrasmall, water-only pores of radius ~2.5 Å present in the endothelial cell membrane (13,14).

2. The three-pore model

The major principles governing solute and fluid transport across the peritoneal membrane are i) diffusion, driven by a concentration gradient according to principles originally described by Adolph Fick (15); and ii) convection, i.e. ultrafiltration driven by osmotic or hydrostatic pressure gradients. Osmosis is a phenomenon that occurs through a barrier restricting the transport of solute compared to that of water. If water is allowed to pass the barrier, while the solute is reflected, then the solute in solution will exert an osmotic pressure across the barrier (19.3 mmHg per 1 mM of solute), promoting water movement into the solution. If the solute is permeable across the barrier, the osmotic force exerted by the solute will be dependent upon the fractional reflection of solute by the barrier, described by the so-called osmotic reflection coefficient (⌠). A ⌠ value of 1.0 denotes the total reflection of solute in the barrier, whereas a ⌠ value of 0 characterizes a fully permeable solute. For small plasma solutes, reflection coefficients across the capillary wall in most continuous capillaries (cf. muscle, fat, connective tissue, peritoneum etc.) have classically been considered to be <0.1 (10).

The continuous endothelium lining the peritoneal capillaries can be functionally described in terms of a three-pore model (TPM) (16,17). In the TPM, approximately 90% of the peritoneal UF-coefficient (L_pS), and 99.5% of the total pore area available for solute transport, is accounted for by small pores, and 5-8% by large pores, occupying <0.5% of the total pore area. Only 2% of the L_pS is accounted for by ultrasmall pores (Fig. 1A, inset). The small pores represent the major transport pathway for small solutes and water in the peritoneal membrane. Across these pores the so-called Starling equilibrium, i.e. the balance between the transcapillary hydrostatic pressure gradient (_P) and the opposing effective colloid osmotic pressure gradient (⌠$_{prot}$_□$_{prot}$; where ⌠$_{prot}$ is ~0.95), is established, where _P normally slightly outweighs ⌠$_{prot}$_□$_{prot}$.

Normally, under non-PD conditions, ~60% of the net capillary UF occurs through small pores, which are largely protein-restrictive, and here _P and ⌠prot_□prot approximately balance out. Across large pores, as much as 40% of the total transcapillary UF occurs, and here _P totally dominates over ⌠prot_□prot, the latter being very low in large pores (because here ⌠prot is low). Only 1-2% of the total peritoneal water transport occurs via the ultrasmall, water-only pores under normal, non-PD, conditions (Fig. 1B). During conditions of PD, fluid removal can be markedly enhanced by the presence of an osmotic agent in the peritoneal cavity. The type of osmotic agent utilized markedly affects the mechanisms of osmosis. Very small osmotic agents (e.g. glycerol, with a Stokes-Einstein (SE)-radius ~3 Å) will exert a low osmotic effect on the small pores, and thereby act primarily on water-only pores (Fig. 1B). Glucose (SE-radius ~3.7 Å) will induce fluid flows, which are about equally partitioned among ultrasmall and small pores. High MW osmotic agents, such as polyglucose (SE-radius ~15-20 Å), will remove fluid mainly *via* the small pores (Fig. 1B). Glucose osmosis will result in a rapid dilution of the peritoneal dialysate due to ultrasmall pore-mediated water flow, as reflected by a fall in dialysate sodium concentration during the first 2 h of the dwell (denoted "sodium sieving"). Furthermore, under PD conditions, the Starling forces "balance" is markedly shifted across the small pores. Indeed, _P has now been markedly reduced, due to the increased intraperitoneal hydrostatic pressure in PD, whereas _□prot has been markedly increased, due to a fall in peritoneal (tissue) colloid osmotic pressure as a consequence of the washout of interstitial proteins by the colloid-free PD fluid. Hence, after the glucose osmotic gradient has dissipated after ~4 h of the dwell, fluid will be reabsorbed through the small pore pathway from the peritoneal cavity to the plasma by the Starling mechanism (18) (Fig. 1B).

Recent research has taken the modeling of peritoneal transport beyond the simple TPM. The simplest extension of the TPM is the so-called serial three-pore membrane/fiber matrix model that includes the interstitial (submesothelial) space as a serial transport resistance acting in concert with the capillary wall (11). More advanced simulations include not only the interstitium but also the distributed nature of the peritoneal capillary barrier in the modeling (19,20). These models exhibit a considerably higher share of capillary aquaporins (⟨c), higher small solute ⌠:s, and a lower peritoneal L_pS than predicted by the original TPM. This model, however, is problematic when it comes to computer simulating the osmotic behavior of oncotic agents, such as Icodextrin. The reason is that the difference in ⌠ between Icodextrin and small solutes (glucose) will then become too low to make Icodextrin osmotically efficient. Furthermore, with the low L_pS of the distributed model, the oncotic (Starling pressure mediated) fluid reabsorption directly into the capillaries will become almost negligible and has to be replaced by a bulk (lymphatic) reabsorption term. Indeed, in a recent computer TPM simulation test, in which the capillary Starling reabsorption of fluid was not specifically accounted for, higher small solute ⌠:s, a higher ⟨c, and a lower value for L_pS than in the original TPM were obtained (21). This is in agreement with theoretical predictions (from the TPM) for the case when the Starling induced capillary fluid reabsorption is artificially diminished, by e.g. reducing the value for L_pS (below 0.06 ml/min/mmHg) (22).

3. Aquaporin-1 is the ultrasmall pore of the peritoneal membrane

The identification of aquaporin-1 (AQP1), a 28 kD protein abundantly expressed in the kidney and red blood cells, as the first member of the aquaporin family (23, for review) had a major relevance for understanding the mechanisms of water transport and ultrafiltration in PD (Fig. 2A-B). Aquaporin-1 is a water-specific membrane channel, impermeable to urea and glycerol, that is distributed in many different cell types including the endothelium lining peritoneal capillaries and post-capillary venules (24). AQP1 is organized as a homotetramer, with each monomer containing six membrane-spanning ⟨-helices forming a right-handed twisted arrangement surrounding a hourglass-shaped central pore. The atomic model of AQP1 (Figure 2C), derived from studies based on electron and X-ray crystallography, revealed key features accounting for the high permeation rate (3 billions water molecules per second) and

the strict selectivity of the channel for water (25). The selectivity of the AQP1 pore is ensured by a narrow constriction of <3 Å diameter (which accommodates a single water molecule, 2.8 Å). Two hydrophilic loops (B and E), each containing a conserved NPA (Asn-Pro-Ala) motif and a short helix, bend into the cavity formed by the six helices. The N termini of these two short ⟨ helices point into the aqueous pore and create a positive electrostatic field that orientates the water molecules to form hydrogen bonds with the two Asn residues (Asn76 and Asn192) that extend their amino groups into the lumen of the channel. This mechanism is breaking the string of hydrogen bonded water molecules through the pore, thus preventing the transport of protons. A transient reorientation of the water dipole occurs at that level, resulting from the hydrogen bonding with the Asn residues. A second constriction involves Arg195, which provides a strong positive charge at the narrowest region of the pore, with His180 and Phe56 (25). The central pore formed by each AQP1 monomer perfectly fits the postulated size of the ultrasmall pore of the peritoneal membrane. Of interest is the location of the side chain of a cysteine residue (Cys189) in the pore, explaining why the water permeability mediated by AQP1 is inhibited by mercury compounds (23).

The functional relevance of AQP1 for PD was first suggested by *ex vivo* inhibition of peritoneal water permeability by $HgCl_2$ in a rat model (13). Studies conducted in the *Aqp1* knock-out (KO) mice demonstrated that the osmotically-driven water transport across the peritoneal membrane was significantly decreased in *Aqp1*$^{-/-}$ mice, as compared with wild-type littermates (26). These data were extended by Ni et al. (14), who used a standard peritoneal exchange test (PET) with hypertonic glucose to demonstrate that AQP1-null mice had a <50% decrease in cumulative ultrafiltration during PD, and no evidence for sodium sieving, confirming the predictions of the TPM (Fig. 3). Heterozygous *Aqp1*$^{+/-}$ mice showed an intermediate phenotype, suggesting that haplo-insufficiency in AQP1 is reflected by significant attenuation of water flux and intraperitoneal volume curves. Of note, the deletion of AQP1 had no effect on the structure of the peritoneal membrane, the transport of small solutes and the magnitude of the osmotic gradient (14).

To date, 13 members of the AQP family have been identified in mammals, with specific expression patterns and distinct roles in given tissues and cells. AQP1 is the most abundant isoform in the peritoneum, and the only one that has been consistently located in the capillary endothelium. It must be noted that the expression of AQP1 in mesothelial, endothelial and epithelial cells may be induced by exposure to osmotic agents *in vitro*, reflecting the presence of hypertonicity response elements in the promoter (27,28). Because the mesothelium does not represent a significant functional barrier for water transport in PD, the functional importance of water channels at that level remains unclear.

4. Aquaporin-1 in ultrafiltration failure

Ultrafiltration failure in long-term PD is usually associated with an increment in small solute transport combined with reductions in the osmotic fluid transport across the peritoneum (11,29). These alterations are not compatible with just an “increased vascular surface area”, i.e. an increased peritoneal vascularization, which would increase both the glucose osmotic efficiency, i.e. the glucose osmotic conductance (product of the peritoneal UF-coefficient, L_pS, and the glucose reflection coefficient, σ_g), and the rate of dissipation of the glucose osmotic gradient across the peritoneum during the dwell (30). By contrast, there seems to be an uncoupling between changes in the mass transfer area coefficient (MTAC) to glucose, and the changes occurring in L_pS in these conditions (11). The unchanging, or even reduced, L_pS in long-term PD has by some authors been attributed to reductions in AQP1-meditated water transport (31). However, a more likely explanation for these changes is that a combination of

two processes, namely i) increases in the vascularization of the peritoneum; and ii) the production of fibrotic scar tissue in the peritoneum, have occurred in parallel (2). It can be shown that a combination of these two processes will actually tend to reduce the osmotic

conductance to glucose (mostly L_pS, but also σ_g) while the MTAC to glucose will tend to increase (due to increases in peritoneal capillary density) (11,30). In fact, overall, there is an increase, and not a reduction, in the abundance of AQP1 in the peritoneal membrane in typical ultrafiltration failure - as pointed in the detailed case study of Goffin et al. (32). It is thus hypothesized that the interstitial fibrosis *per se* accounts for the reduction in transperitoneal UF, across both small and ultrasmall pores *via* a reduction in total L_pS (31-33). One should stress, however, that mechanisms of ultrafiltration failure probably differ in shortvs. long-term PD patients (31) and may also include rare instances of increased absorption of fluid from the peritoneal cavity (34).

5. Regulation of aquaporin-1 expression to increase ultrafiltration

The fact that AQP1 is the molecular counterpart of the ultrasmall pore, mediating half of the ultrafiltration volume during crystalloid osmosis, suggested that it could be a target to modulate water transport in PD. The proof-of-principle for that concept was provided by a study in a rat model of PD (35). Knowing that the expression of AQP1 in the perinatal rat lung is induced by corticosteroids and that the promoter of the *AQP1* gene contains glucocorticoid response elements (GREs), Stoenoiu et al. showed that a treatment of rats with dexamethasone (1 to 4 mg/kg BW) for 5 days was associated with an increased expression of AQP1 in the capillary endothelium. In turn, this induction was reflected by a significant increase in water transport and net ultrafiltration across the membrane. These modifications were observed in absence of any effect on the osmotic gradient or small solute transport (35). More recently, de Arteaga et al. reported serial determinations of water and solute transport parameters across the peritoneal membrane (mini PET) in three PD patients that received high-dose glucocorticoids (cumulative dose of methylprednisolone: 1.0–1.2 g/m^2) as standard care after a living-donor renal transplantation (36). As compared with pre-transplantation, the post-transplantation PET evidenced an ~2-fold increase in the sodium sieving (and its surrogate marker the absolute dip of dialysate sodium concentration), paralleled by an increase in the ultrasmall pore-specific UF. In contrast, the small solute transport rate, as assessed by the D/P creatinine and small pore-specific ultrafiltration, was unchanged. The potential role of the GRE-mediated effect on AQP1 was substantiated by demonstrating the expression of the glucorticoid receptor in the capillary endothelium of the human peritoneum (36). These data were the first to support the effect of corticosteroids on AQP1-mediated water transport in PD patients. Although these results have to be confirmed in a larger patient cohort and take into account the systemic effects of high-dose steroid administration, the specificity of the changes in free water transport suggests that pharmacologic induction of AQP1 may help to increase water transport in patients treated by PD. Future investigations should provide a better understanding of the regulators of AQP1 expression, the time-course of such regulation, and the potential effect of such regulators in different cell types expressing AQP1 (2).

6. Pharmacological agonists of the aquaporins

Beyond the possibility to induce the expression of AQP1 in peritoneal capillaries, an equally exciting opportunity is the search for pharmacological agonists of aquaporins, which would bind reversibly to the channel to further facilitate water transport. This possibility implies that aquaporins can be gated, i.e. they are not constitutively locked into a rigid open state. For instance, the lens water channel AQP0 as well as plant aquaporins show changes in water permeability as a function of pH (37,38). By extension, some aquaporins may have both open and closed state conformations, with overall water permeability at a given time reflecting the relative proportion of the channels in the open state. Stabilization of the open state conformation by ligand binding would then yield agonist activity.

Mechanisms of gating of aquaporin channels are being defined. Intracellular domains

have been proposed to gate channel functions in AQP1 (39,40) and the closely related AQP4 (41). Aquaporins are highly conserved in animals and plants. A tight regulation of their aquaporins allows plants to cope with fluctuations in water supply during drought stress or flooding. Tornroth-Horsefield and colleagues used structural modeling to outline a detailed mechanism of gating in a spinach plasma membrane aquaporin (42). In this model, loop D is a hydrophobic barrier that caps and occludes the intracellular side of the water pore, in response to dephosphorylation of conserved serine residues or, alternatively, to the protonation of a conserved histidine (flooding stress). Interaction with the N-terminal domain and Ca^{2+} binding help to anchor loop D into the cytoplasmic pore vestibule, occluding the water channel. In the open conformation, the loop D is displaced after phosphorylation of the conserved serine residues, which induces rearrangement of residues that coordinate Ca^{2+} ion binding, disrupting the stabilizing interaction between loop D and the N terminus, and opening the hydrophobic gate (42).

Modulators of AQP1 have been slow to emerge. Antagonists of AQP1 and AQP4 have been described, with a focus on arylsulfonamide compounds including the carbonic anhydrase inhibitor acetazolamide (43) and derivatives of the loop diuretic bumetanide (44). Of particular interest, a 4-aminopyridine carboxamide derivative of the loop diuretic bumetanide (AqB013) was shown to inhibit AQP1 *in vitro* by acting at an intracellular side that occludes the water pore (44). Docking simulations have identified two potential intracellular binding sites in AQP4, one being positioned in the water pore vestibule whereas the second is in a separate intracellular pocket that involves residues from the C-terminus and intracellular loop (44). This second site, which has a potential for allosteric regulation of the pore, has also been associated with regulation of AQP1 by cGMP (39), protein kinase C (40) and Ca^{2+} (45).

Recent work has identified the first known aquaporin pharmacological agonist, AqF026, a chemical derivative of the arylsulfonamide compound furosemide (46) (Fig. 4A). In the *Xenopus laevis* oocyte system, extracellularly applied AqF026 at 5-20 ⌈M potentiated water channel activity of human AQP1 by more than 20%, but had no effect on AQP4 water channel activity. The intracellular binding site for AQP1, predicted by theoretical docking and tested by site-directed mutagenesis and swelling assays, involves the loop D which is known to be associated with channel gating. *In vivo* assays in a mouse model of PD (Fig. 4B-D) confirmed that AqF026 enhanced the osmotic water transport and net ultrafiltration across the peritoneal membrane without detectable effect on the osmotic gradient, the transport of small solutes, and the plasma membrane localization and expression levels of AQP1. Absence of potentiation effects of AqF026 in *Aqp1*-null mice further indicated that indirect mechanisms involving other channels or transporters were unlikely. The first identification of an aquaporin agonist able to increase osmotic water transport *in vivo* offers perspectives for PD and clinical situations associated with defective water handling (46).

Conclusions and perspectives

Transcellular water permeability mediated by AQP1 is an essential component of the water removal across the peritoneal membrane during peritoneal dialysis. Several lines of evidence have demonstrated that AQP1 is the ultrasmall, water-specific pore predicted by the TPM. Studies in the AQP1 KO mice demonstrated a strict correlation between AQP1 abundance and solute-free water transport across the peritoneal membrane. They also confirmed that AQP1 is responsible for approximately 50% of the ultrafiltration when using a crystalloid osmotic agent such as glucose, and that AQP1 expression is necessary to observe the sodium sieving (14). By extension, the availability of the *Aqp1* mouse model is useful to test the properties of alternative osmotic agents, as recently demonstrated for L-carnitine (47), or combinations of such agents.

The studies outlined above support the validity of the TPM, which can now be tested against a simple and validated mouse model (48) allowing direct measurement of free-water transport in conditions of modified expression and, potentially, gating of AQP1. Assessing water transport across the peritoneum has already been shown to be useful for *in vivo*

investigations of drugs or molecular pathways regulating AQP1 (35,36,47). Whether biochemical modifications of AQP1 (e.g. S-nitrosylation or abnormal glycation) may alter the integrity of the water pore and contribute to ultrafiltration failure remains unsolved (31,32). Similarly, the potential influence of variants in the *AQP1* gene encoding AQP1 in the individual variability in free-water transport at the start of PD remains to be defined.
The identification of the ultrasmall pore offers the opportunity to modulate water transport in PD. Proof-of-principle studies in rat and mouse models (35,46) and in PD patients (36) have shown that such modulation can be achieved without modifying the osmotic gradient or the small solute transport. Whether increasing the expression of AQP1 in the peritoneal membrane or gating the already existing channels could be used to treat UF failure and overhydration in some PD patients remains to be investigated. The maximal increase of AQP1-mediated free-water transport will occur during the first part of the dwell, when the osmotic gradient is maximal. A potentiation of AQP1 could thus be transient (lasting a few hours in the dwell), but may be important in patients treated with short dwells. Such a benefit could be hampered by the lack of sodium removal in multiple, high tonicity rapid exchanges. However, combining an AQP agonist with a low sodium solution might resolve this potential issue. The translational potential of agonists of AQP1 should also integrate the potential role of water channels in endothelial cell proliferation (49).

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LEGENDS TO FIGURES

Figure 1. Structure of the peritoneal membrane and the three-pore model.

A. Cross-section of the human parietal peritoneum stained for the water channel aquaporin-1. The peritoneal membrane contains three components : a layer of ciliated mesothelial cells (*), with microvilli and apical protrusions into the peritoneal cavity (#); the interstitial tissue containing bundles of collagen and mucopolysaccharides; a dense network of capillaries, blood vessels, and lymphatics. During peritoneal dialysis, the microvascular endothelium (arrows, stained in red) represents the functional barrier for the transport of solutes and water from the blood of the patient to the dialysate that has been instilled in the peritoneal cavity.
Inset. The continuous endothelium lining the peritoneal capillaries can be functionally described as a three-pore model. The small pores (radius ~40-50 Å), located between the endothelial cells account for approximately 90% of the peritoneal UF-coefficient (L_pS, or hydraulic conductance), and 99.5% of the total pore area available for solute transport. The large pores (radius ~250 Å), thought to correspond to interendothelial gaps, account for 5-8% of the LpS, occupying <0.5% of the total pore area. The ultrasmall pores (radius ~2.5 Å), wich account for only 2% of the L_pS are the only ones to be located in the endothelial cells. The Starling forces (P, hydrostatic pressure; ∠, oncotic pressure) operating across each type of pore are indicated. r, functional radius; Å, angström (10^{-10} m).
B. Transcapillary ultrafiltration in the three-pore model.
a. Fractional fluid flows across the peritoneum under normal conditions with no dialysis. In absence of osmotic agent, ~60% of the transcapillary fluid flow occurs through small pores, where the Starling forces are close to equilibrium. Approximately 40% of the capillary UF occurs across large pores where there is hardly any colloid osmotic pressure counteracting the transcapillary hydrostatic pressure gradient.
b. With glycerol (SE-radius ~3 Å) as osmotic agent, ~55% of the transperitoneal water flow will occur through water-only, ultrasmall pores and 45% through small pores. This is due to the relative inefficiency of glycerol as osmotic agent across the small pore pathway (σ ~0.02).
c. With glucose (SE-radius ~3.7 Å) as osmotic agent ~45% of the transperitoneal water flow will occur through water-only pores and 55% through small pores. Although glucose is a relatively inefficient osmotic agent in the small pore pathway (σ ~0.03), it is 50% more efficient than glycerol.
d. In a conventional Icodextrin PD solution, ~25-30% of the molecules (~3 mM) act as a colloid, implying a reflection coefficient close to unity. It should be noted that 3 mM of high

MW icodextrin will produce a colloid osmotic pressure of ~58 mmHg (3 x 19.3 mmHg), which is sufficient to counteract the plasma colloid osmotic pressure (22-26 mmHg) exerted by approximately 1 mM of negatively charged plasma proteins. Note, that the partitioning of fluid flows among the different porous pathways in the TPM is now almost identical to that in the peritoneum occurring during high net UF conditions in the absence of crystalloid osmotic forces.

e. Reabsorption of fluid across the small pores occurs when the crystalloid (glucose) osmotic gradient has totally dissipated (usually after 4 h). The net Starling fluid balance is biased toward reabsorption across the small pores in PD. Some fluid reabsorption will also occur across AQP1. Minute ultrafiltration still occurs across the large pores.

Figure 2. Expression and distribution of AQP1 in the peritoneal membrane; structure of the water pore.

A. Immunoblotting for AQP1 in human kidney (HK) and human peritoneal membrane (HPM) extracts. The signal for the core (28 kD) and glycosylated (35-50 kD) AQP1 is detected in the extracts, with lower expression levels in the peritoneal membrane.

B. Immunogold electron microscopy on mouse visceral peritoneum unicryl sections shows a very strong signal for AQP1 in the plasma membrane and plasma membrane infoldings of capillary endothelial cells. Comparison of the labeling density in wild-type mice shows that AQP1 labeling is markedly stronger in endothelial cells than in red blood cells. (Bar, 500 nm).

C. Structure and selectivity of the water pore of AQP1. The channel consists of an extracellular and a cytoplasmic vestibule connected by an extended narrow pore or selectivity filter. Four bound water molecules are localized within the selectivity filter, along three hydrophilic binding sites in the long hydrophobic pore segment. Residues of the constriction region, in particular Arg195, His180, Asn192 and Asn76, are critical in establishing water specificity and proton exclusion. For details, see text. (Modified from Ref. #25).

Figure 3. Effect of AQP1 deletion on the transport of water across the peritoneal membrane.

Mice with a targeted deletion of *Aqp1* were investigated using a peritoneal equilibration test using hypertonic glucose. The cumulative ultrafiltration (2h-dwell, 7% glucose Dianeal®) is halved in AQP1-null mice vs. wild-type controls **(A)**. In comparison with wild-type mice (squares), mice lacking AQP1 (circles) show a complete loss of sodium sieving **(B)**. The intraperitoneal volume vs. time ($V_{(t)}$) curves (**C**, obtained with 2.5 ml of 3.86% glucose Dianeal® containing 50 ⌈l of 10% bovine serum albumin and 50 ⌈l of ^{125}I-human serum albumin) were also significantly lower in the *Aqp1*$^{-/-}$ mice (circles) compared to *Aqp1*$^{+/+}$ mice (squares). Intermediate values of sodium sieving and intraperitoneal volume curves are observed in heterozygous *Aqp1*$^{+/-}$ mice. There were at least 5 mice matched for age and gender in each group. *P<0.05 vs Aqp1+/+ mice and #P<0.05 vs Aqp1+/- mice.
(Modified from Ref. #14).

Figure 4. AqF026, a novel agonist of AQP1, increases osmotic water transport *in vivo.*

A. Chemical structure of AqF026 and the parent compound furosemide.

B. Wild-type *Aqp1*$^{+/+}$ mice treated with AqF026 have a significant, dose-dependent increase in net ultrafiltration (UF) across the peritoneal membrane. The maximal response is observed for a concentration of 15 μM, with an estimated EC50 value of 4.2 μM (*inset*). *Aqp1*$^{-/-}$ mice, characterized by a 60% reduction in UF at baseline, show no potentiation of the UF after treatment with 15 mM AqF026. Data are mean ± SEM; n=6 for each AqF026 concentration except for 0.75 μM (n=4). Net UF rates were standardised to body weight and compared with vehicle-treated mice (open bar).

C. Treatment of *Aqp1*
$^{+/+}$ mice with 15 μM AqF026 results in increased intraperitoneal (IP)
volume over time (p=0.016 between the AqF026 and vehicle curves), with significant differences at the 30, 60 and 90 min time-points in AqF026- (black triangles), as compared with vehicle-treated (open squares) animals (p<0.01, p<0.05 and p<0.05 respectively; n=10 in each group).

D. The dialysate-to-plasma ratio of osmolality at 30 min (D/P osm) was similar in mice treated with 15mM AqF026 vs. vehicle (n=12 pairs of *Aqp1*$^{+/+}$ mice).
Data compiled from Ref 46, with permission.